Claims

1. A peptide having natriuretic activity of the formula:

$$R^1\text{-Cys-Phe-Gly-Arg-}\begin{matrix}\text{Arg/}\\\text{Lys}\end{matrix}\text{-}\begin{matrix}\text{Leu/}\\\text{Met}\end{matrix}\text{-Asp-Arg-Ile-}\begin{matrix}\text{Gly/}\\\text{Ser}\end{matrix}\text{-}\begin{matrix}\text{Ser-}\\\text{Ser}\end{matrix}\text{-Ser-Gly-Leu-Gly-Cys-}R^2 \quad (1)$$

wherein R^1 is selected from the group consisting of:

(H);

Gly-;

Ser-Gly-;

$\begin{matrix}\text{Asp/}\\\text{Lys/}\\\text{Gly}\end{matrix}$-Ser-Gly-;

$\begin{matrix}\text{Arg/}\\\text{His/}\\\text{Gln}\end{matrix}$-$\begin{matrix}\text{Asp/}\\\text{Lys/}\\\text{Gly}\end{matrix}$-Ser-Gly-;

$\begin{matrix}\text{Met/}\\\text{Val}\end{matrix}$-$\begin{matrix}\text{Arg/}\\\text{His/}\\\text{Gln}\end{matrix}$-$\begin{matrix}\text{Asp/}\\\text{Lys/}\\\text{Gly}\end{matrix}$-Ser-Gly-;

$\begin{matrix}\text{Thr/}\\\text{Met}\end{matrix}$-$\begin{matrix}\text{Met/}\\\text{Val}\end{matrix}$-$\begin{matrix}\text{Arg/}\\\text{His/}\\\text{Gln}\end{matrix}$-$\begin{matrix}\text{Asp/}\\\text{Lys/}\\\text{Gly}\end{matrix}$-Ser-Gly-;

Lys-$\begin{matrix}\text{Thr/}\\\text{Met}\end{matrix}$-$\begin{matrix}\text{Met/}\\\text{Val}\end{matrix}$-$\begin{matrix}\text{Arg/}\\\text{His/}\\\text{Gln}\end{matrix}$-$\begin{matrix}\text{Asp/}\\\text{Lys/}\\\text{Gly}\end{matrix}$-Ser-Gly-;

Pro-Lys-$\begin{matrix}\text{Thr/}\\\text{Met}\end{matrix}$-$\begin{matrix}\text{Met/}\\\text{Val}\end{matrix}$-$\begin{matrix}\text{Arg/}\\\text{His/}\\\text{Gln}\end{matrix}$-$\begin{matrix}\text{Asp/}\\\text{Lys/}\\\text{Gly}\end{matrix}$-Ser-Gly-;

Ser-Pro-Lys-(Thr/Met) - (Met/Val) - (Arg/His/Gln) - (Asp/Lys/Gly) -Ser-Gly-;

or a 10- to 109-amino acid sequence shown as the native upstream sequence for porcine, canine or human BNP in Figure 8, or a composite thereof;

R^2 is (OH), NH_2, or NR'R" wherein R' and R" are independently lower alkyl (1-4C) or is

(Asn/Lys)

(Asn/Lys) -Val

(Asn/Lys) -Val-Leu

(Asn/Lys) -Val-Leu-Arg

(Asn/Lys) -Val-Leu-Arg- (Arg/Lys)

(Asn/Lys) -Val-Leu-Arg- (Arg/Lys) - (Tyr/His)

or the amides (NH_2 or NR'R") thereof,

with the proviso that if formula (1) is

R^1-Cys-Phe-Gly-Arg-Arg-Leu-Asp-Arg-Ile-Gly-Ser-Leu-Ser-Gly-Leu-Gly-Cys-R^2

and R^1 is Asp-Ser-Gly-, R^2 cannot be Asn-Val-Leu-Arg-Arg-Tyr.

2. A peptide having natriuretic activity which is a modified form of the peptide of claim 1, having conservative amino acid substitutions in one or two positions.

3. The peptide of claim 1 having the formula:

R^1-Cys-Phe-Gly-Arg-Arg-Leu-Asp-Arg-
Ile-Gly-Ser-Leu-Ser-Gly-Leu-Gly-Cys-R^2

wherein R^1 is selected from Thr-Met-Arg-Asp-Ser-Gly; Ser-Pro-Lys-Thr-Met-Arg-Asp-Ser-Gly; and Gly-Ile-Arg-Ser-Pro-Lys-Thr-Met-Arg-Asp-Ser-Gly; and the 10- to 108-amino acid upstream sequence shown for porcine prepro-BNP in Figure 8.

4. The peptide of claim 1 having the formula

R^1-Cys-Phe-Gly-Arg-Arg-Leu-Asp-Arg-
Ile-Gly-Ser-Leu-Ser-Gly-Leu-Gly-Cys-R^2

wherein R^1 is selected from

(H);
Lys-Ser-Gly-;
His-Lys-Ser-Gly-;
Met-His-Lys-Ser-Gly-;
Met-Met-His-Lys-Ser-Gly-;
Lys-Met-Met-His-Lys-Ser-Gly-;
Pro-Lys-Met-Met-His-Lys-Ser-Gly-;
Ser-Pro-Lys-Met-Met-His-Lys-Ser-Gly-;

and the 10- to 109-amino acid sequence shown for canine BNP in Figure 8.

5. The peptide of claim 1 having the formula

R^1-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-
Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-R^2.

6. The peptide of claim 5 wherein R^1 is selected from

(H);
Gly-;
Ser-Gly-;
Gly-Ser-Gly-;

Gln-Gly-Ser-Gly-;
Val-Gln-Gly-Ser-Gly-;
Met-Val-Gln-Gly-Ser-Gly-;
Lys-Met-Val-Gln-Gly-Ser-Gly-;
Pro-Lys-Met-Val-Gln-Gly-Ser-Gly-;
Ser-Pro-Lys-Met-Met-His-Lys-Ser-Gly-;

and the 10- to 109-amino acid sequence shown for human BNP in Figure 8.

7. A recombinant DNA in isolated form consisting essentially of a DNA which encodes the peptide of claim 1.

8. A recombinant expression system capable, when contained in a recombinant host cell, of expressing the DNA encoding the peptide of claim 1.

9. A recombinant host cell or cell culture which has been manipulated so as to contain the expression system of claim 8.

10. A method to produce a peptide having natriuretic activity, which method comprises:

culturing the cells of claim 9 under conditions which permit the expression of the DNA encoding said peptide; and

recovering the peptide from the culture.

11. A pharmaceutical composition useful in treating conditions associated with a high level of extracellular fluid which composition comprises an effective amount of the peptide of claim 1 in admixture with a suitable pharmaceutical excipient.

12. A method to treat a condition characterized by a high level of extracellular fluid which comprises administering to a subject in need of treatment an effec-

tive amount of the peptide of claim 1 or a pharmaceutical composition thereof peptide.

13. The method of claim 12 wherein the condition is congestive heart failure.

14. A pharmaceutical composition useful in treating conditions associated with a high level of extracellular fluid which composition comprises an effective amount of the peptide of claim 6 in admixture with a suitable pharmaceutical excipient.

15. A method to treat a condition characterized by a high level of extracellular fluid which comprises administering to a subject in need of treatment an effective amount of the peptide of claim 6 or a pharmaceutical composition thereof.

16. The method of claim 15 wherein the condition is congestive heart failure.

17. A DNA probe useful in retrieving the gene encoding brain natriuretic peptide from a vertebrate DNA library, which probe comprises the pBNP-encoding cDNA shown in Figure 1, or an effective portion thereof.

18. A DNA sequence encoding a peptide having natriuretic activity, which DNA hybridizes directly or indirectly with the probe of claim 17 at 42°C in buffer containing 20% formamide, 5 x Denhardt's, 6 x SSC, 100 mg/ml RNA, 0.05% Na pyrophosphate, followed by washing at 60°C at 1 x SSC, 0.1% SDS.

19. A peptide having natriuretic activity encoded by a DNA, which DNA hybridizes directly or in-

directly with the probe of claim 17 at 42°C in buffer containing 20% formamide, 5 x Denhardt's, 6 x SSC, 100 mg/ml RNA, 0.05% Na pyrophosphate, followed by washing at 60°C at 1 x SSC, 0.1% SDS,

with the proviso that said peptide is not pBNP.

20. A DNA sequence which comprises a segment of contiguous or non-contiguous portions of the cDNA of Figure 1, which segment encodes a brain natriuretic peptide having natriuretic activity.

21. The DNA of claim 20 wherein the segment encodes pBNP.

22. The DNA of claim 20 wherein the segment encodes the peptide sequence Thr-Met-Arg-pBNP.

23. The DNA of claim 20 wherein the segment encodes the peptide sequence Ser-Pro-Lys-Thr-Met-Arg-pBNP.

24. The DNA of claim 20 wherein the segment encodes the peptide sequence Gly-Ile-Arg-Ser-Pro-Lys-Thr-Met-Arg-pBNP.

25. A DNA sequence which comprises a modified segment of contiguous or non-contiguous portions of the cDNA of Figure 1 wherein said modified segment encodes a BNP having one or two conservative amino acid substitutions.

26. A recombinant expression system which comprises the segment of claim 20 operably linked to control sequences functional in a recombinant host.

27. A recombinant host transformed with the expression system of claim 26.

28. A method to produce a recombinant brain natriuretic peptide which comprises culturing the transformed host of claim 27 under conditions suitable for the expression of the BNP and,

recovering BNP from the culture.

29. Recombinant BNP produced by the method of claim 28, with the proviso that said BNP is not pBNP.

30. A brain natriuretic peptide which is a modification of a BNP encoded by a segment of contiguous or non-contiguous portions of the cDNA of Figure 1 wherein one or two positions contain conservative amino acid substitutions.

31. The BNP of claim 30 wherein one of said substitutions is substitution of the D- for the L-form.

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